EXHIBIT 13

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Clinical Protocol

Immunotherapy for Cancer by Direct Gene Transfer into Tumors

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1.0 Introduction

1.1 Rationale and Relationship to Previous Work

This proposal represents an extension of a previously approved protocol submitted and approved by the Recombinant DNA Advisory Committee on February 10, 1992, entitled "Immunotherapy of Malignancy by *In Vivo* Gene Transfer into Tumors." Since the implementation of the first protocol, there have been four developments which have led to the modifications proposed in this study. The background and description of the previous trial is included in the appendix (1–5). The previous trial has now been completed, and the conclusions have been summarized in the accompanying data. Briefly, the trial has demonstrated that recombinant gene expression can be achieved following direct gene transfer into melanoma in humans. In addition, there has been no evidence of toxicity from this treatment, and no antibody responses were detected to DNA as a result of this intervention. The clinical and immunologic responses are currently undergoing evaluation, and the status of these studies will be summarized at the RAC meeting; however, based on the findings of gene expression and lack of toxicity, we have felt that it is appropriate to pursue this general approach to the introduction of a foreign antigen into tumors in an effort to stimulate anti-tumor immunity.

Since the initial trial, there have been advances in four areas of the gene transfer technology. In this protocol, we will incorporate these improvements. Briefly, these improvements include:

1) development of a more efficacious cationic liposome which improves efficiency of gene delivery
2) improvements in vector design which further enhance expression *in vivo*
3) inclusion of catheter-based gene delivery
4) application to different tumor cell types

The preliminary data regarding each of these developments is shown in Section 13 (Preliminary Data). Briefly, a new formulation of cationic lipids has been described recently by Dr. Phillip Felgner in which a different cationic lipid, 1,2-dimyristyloxypropyl-3-dimethyl-hydroxyethyl ammonium bromide (DMRIE), is utilized with dioleoyl phosphatidylethanolamine (DOPE). This has two properties which make it more suitable for these studies. First, it shows up to 10-fold improved transfection efficiency compared to our previous formulation, DC-chol, *in vitro*. More importantly, this formulation does not aggregate at high concentrations, in contrast to the DC-Chol liposome. This characteristic thus allows higher absolute con-

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centrations of DNA and liposomes to be introduced into experimental animals without toxicity (Section 13, Preliminary Data). Because of these properties, it now becomes possible to introduce 100–1000 times more DNA which could markedly improve gene expression *in vivo* (Section 13, Preliminary Data).

The vector improvements are divided into two categories for this proposal. In the first case, expression of the HLA-B7 vector has been improved by the addition of a consensus translation initiation sequence and removal of an intron. In addition, the inclusion of the β-2 microglobulin gene, with which class I MHC genes normally associate, allows synthesis of the complete histocompatibility molecule, which is composed of these two chains. Ordinarily, these two gene products are co-transported to the cell surface, and some human melanoma cells do not express endogenous β-2 microglobulin, thus limiting their ability to stably express class I on the cell surface. We have found that the inclusion of the β-2 microglobulin gene on the same plasmid allows for the expression in these otherwise resistant cells and improves expression in other cells, thus overcoming a potential mechanism of resistance. A potential future modification of the vector involves the expression of a cytokine gene in addition to class I MHC and β-2 microglobulin. The elaboration of cytokines such as IL-2 and GM-CSF could further stimulate T cell immunity against the tumor locally and improve recognition of tumor-associated antigens. In experimental animal models, the introduction of IL-2 has allowed for improved antitumor efficacy ((6); see Section 13, Preliminary Data). To maximize the safety concerns for vectors which produce cytokines in the future, we would include the cytokine gene on the same transcript as the class I MHC gene, linking the expression of the cytokine gene to expression of the foreign histocompatibility antigen. In this way, cells which contain the cytokine gene would be eliminated after several weeks *in vivo*, minimizing any concerns regarding persistent expression of cytokines *in vivo*. At present, such a vector is not yet available; however, we would ask to include it in the future if it proves more effective than previous vectors.

The third amendment to the protocol is the delivery of recombinant genes using a catheter. This modification was proposed in our previous trial as a minor amendment for one patient in the protocol with a pulmonary metastases. This treatment was well-tolerated by the patient. No complications or toxicities were noted (summarized in Section 13, Preliminary Data, Table 3). In this trial, we propose to analyze the toxicity associated with catheter-based gene delivery separately, since the toxicities of this procedure may differ from direct intratumor injection. This intervention provides the ability to transduce a larger percentage of cells within the tumor microcirculation in order to achieve greater efficacy of gene expression, at the same time minimizing the potential for inadvertent microscopic seeding of tumor cells to distant sites. Because this approach is not always feasible, we have also elected to continue the direct intratumoral injection approach. Finally, we have shown previously that this method of direct gene transfer is applicable to different tumor cell types. Particularly, for the *in vivo* catheter delivery, it may be appropriate to utilize this approach on malignancies other than melanoma. We therefore would like to maintain the option to treat other human cancers, for example, colon carcinoma, renal cell carcinoma, or breast cancer, in this study. These cancers would most likely be treated by catheter delivery.

Because of these several improvements, we have submitted a protocol which proposes to incorporate these modifications in another phase I/II clinical trial to determine the safety and toxicity of this treatment. The study design and clinical endpoints remain almost identical to the previous trial, and are detailed in the following sections.

1.2 Background

A variety of genetic abnormalities arise in human cancer which contribute to neoplastic transformation and malignancy. Instability of the genome generates mutations which alter cell proliferation, angiogenesis, metastasis, and tumor immunogenicity. Despite a better understanding of the molecular basis of cancer, many malignancies remain resistant to traditional forms of treatment. The definition of tumor-associated genetic mutations, however, has heightened interest in cancer as a target for gene therapy. Immunotherapy has shown promise as a primary approach to the treatment of malignancy. Indeed, specific cancers, such as melanoma or renal cell carcinoma, are relatively more responsive to modulation of immune function, possibly because the immune system can be induced to recognize mutant gene products in these cells. Conventionally, approaches to immunotherapy have involved the administration of non-specific immunomodulating agents such as Bacillus Calmette-Guerin (BCG), cytokines, and/or adoptive T cell transfer, which have shown promise in animal models (7–12) and in man (13–16). More recently, molecular genetic interventions have been designed in an attempt to improve the efficacy of immunotherapy. Human gene transfer protocols have been designed to monitor the traffic of lymphocytes into melanoma tumors (17) or to introduce cytokine genes into tumor cells to stimulate the host's immune response to residual tumor (18).

We have recently developed a novel molecular genetic intervention for human malignancy. This approach relies on the direct transmission of recombinant genes into established tumors *in vivo* to genetically modify them as they grow *in situ*. In animal models, introduction of a gene encoding a foreign major histocompatibility (MHC) protein (class I) *in vivo* signals the immune system to respond to the foreign antigen (5,19). More importantly, when this gene is transduced into established tumors *in vivo*, a cytolytic T cell response is also generated against unmodified tumor cells. In murine models, this approach has led to significant reductions in tumor growth and, in some cases, complete remission (5). Based on these studies, we received approval from the Recombinant DNA Advisory Committee of the National Institutes of Health to conduct a human clinical protocol using direct transfer of a human transplantation antigen gene in an effort to treat malignancy. This protocol proposed to perform direct gene transfer in humans and to utilize a non-viral vector which reduces several safety concerns about viral vectors. This clinical trial involved the treatment of patients with metastatic melanoma at subcutaneous lesions. The treatment consists of intratumoral injection of the human class I MHC gene, HLA-B7 complex, and a cationic liposome, DC-Cholesterol (2,20). These patients received escalating doses of the DNA liposome complex. Recombinant gene expression, toxicity, and the immunologic response to treatment is being evaluated. The objectives of this first human clinical trial using direct gene transfer were (1) to establish a safe and effective

dose to introduce recombinant genes *in vivo*, (2) to confirm expression of the transduced gene, and (3) to analyze the immune response and potential therapeutic effects of this method of therapy. Based on animal studies, no toxicities had been readily apparent using these modes of direct gene transfer *in vivo* in short-term or long-term studies (1–3). Taken together, these studies were intended to determine whether direct gene transfer was an appropriate form of treatment for malignancy. In addition, the data compiled from this study also provided an indication of whether this method of delivery could be appropriate for the treatment of a variety of other human diseases.

In this study, another phase I clinical trial is proposed. The safety of this method and appropriate dosage in humans will be tested. Recombinant gene expression *in vivo* will be confirmed, and the specificity and mechanism of immune rejection will be defined. In subsequent phases, this response will be augmented by preimmunization and administration of cytokines, including tumor necrosis factor-α, interferon-γ, or interleukin-2, or used in combination with adoptive transfer or TIL therapy. These studies will provide an alternative strategy for the immunotherapy of malignancy and allow definition of the mechanism of immune rejection of tumor cells. Adaptations of this method may also eventually be applied to the treatment of other human diseases.

1.3 Direct Gene Transfer and Modulation of the Immune System

The utilization of catheter-based gene delivery *in vivo* provided a model system for the introduction of recombinant gene-specific sites *in vivo*. Early studies focused on the demonstration that specific reporter genes could be expressed *in vivo* (21,22). Subsequent studies were designed to determine whether specific biologic responses could be induced at sites of recombinant gene transfer. To address this question, a highly immunogenic molecule, a foreign major histocompatibility complex (MHC), was used to elicit an immune response in the iliofemoral artery using a porcine model. The human HLA-B7 gene was introduced using direct gene transfer with a retroviral vector or DNA liposome complex (19). With either delivery system, expression of the recombinant HLA-B7 gene product could be demonstrated at specific sites within the vessel wall. More importantly, the expression of this foreign histocompatibility antigen induced an immunologic response at the sites of genetic modification. This response included a granulomatous mononuclear cell infiltrate beginning 10 days after introduction of the recombinant gene. This response resolved by 75 days after gene transfer; however, a specific cytolytic T cell response against the HLA-B7 molecule was persistent. This study demonstrated that a specific immunologic response could be induced by the introduction of a foreign recombinant gene at a specific site *in vivo*. Moreover, this provided one of the first indications that direct gene transfer of specific recombinant genes could elicit an immune response to the product of that gene *in vivo* (19).

These studies suggested that the introduction of the appropriate recombinant genes could be used to stimulate the immune system to recognize its product *in vivo*. In addition, this approach provided a general method for the induction of a specific site *in vivo*. To determine whether direct gene transfer might be appropriate for the treatment of disease, a murine model of malignancy has been developed. Direct gene transfer of an allogeneic histocompatibility complex gene into a murine tumor elicits an immune response not only to the foreign MHC gene but also to previously unrecognized tumor-associated antigens. These immune responses are T cell-dependent, and these tumor-associated proteins are recognized within the context of the self major histocompatibility complex. In animals presensitized to a specific MHC haplotype, direct gene transfer into established tumors could attenuate tumor growth or, in some cases, lead to complete tumor regression (5). These studies demonstrate that direct gene transfer of foreign MHC genes into tumors have potentially therapeutic effects that may be appropriate for the treatment of malignancy.

1.4 Immunotherapy of Malignancy

In some instances, the immune system appears to contribute to the surveillance and destruction of neoplastic cells, either by mobilization of cellular or humoral immune effectors. Cellular mediators of anti-tumor activity include MHC-restricted cytotoxic T cells, natural killer (NK) cells (23,24) and lymphokine-activated killer (LAK) cells (25). Cytolytic T cells which infiltrate tumors have been isolated and characterized (26). These tumor infiltrating lymphocytes (TIL) selectively lyse cells of the tumor from which they were derived (10,27). Macrophages can also kill neoplastic cells through antibody-dependent mechanisms (28,29), or by activation induced by substances such as BCG (30).

Cytokines can also participate in the anti-tumor response, either by a direct action on cell growth or by activating cellular immunity. The cytostatic effects of tumor necrosis factor-α (TNF-α) (31) and lymphotoxin (32) can result in neoplastic cell death. Interferon-γ (IFN-γ) markedly increases class I MHC cell surface expression (33,34) and synergizes with TNF-α in producing this effect (35). Colony stimulating factors such as G-CSF and GM-CSF activate neutrophils and macrophages to lyse tumor cells directly (36), and interleukin-2 (IL-2) activates Leu-19+ NK cells to generate lymphokine activated killer cells (LAK) capable of lysing autologous, syngeneic or allogeneic tumor cells, but not normal cells (25,37,38). The LAK cells lyse tumor cells without preimmunization or MHC restriction (39). Interleukin-4 (IL-4) also generates LAK cells and acts synergistically with IL-2 in the generation of tumor specific killer cells (40).

Since most malignancies arise in immunocompetent hosts, it is likely that tumor cells have evolved mechanisms to escape host defenses, perhaps through evolution of successively less immunogenic clones (41). Deficient expression of class I MHC molecules limits the ability of tumor cells to present antigens to cytotoxic T cells. Freshly isolated cells from naturally occurring tumors frequently lack class I MHC antigen completely or show decreased expression (42–46). Reduced class I MHC expression could also facilitate growth of these tumors when transplanted into syngeneic recipients. Several tumor cell lines which exhibit low levels of class I MHC proteins become less oncogenic when expression vectors encoding the relevant class I MHC antigen are introduced into them (47–51). In some experiments, tumor cells which express a class I MHC gene confer immunity in naive recipients against the parental tumor

(48,49). The absolute level of class I MHC expression however, is not the only factor which influences the tumorigenicity or immunogenicity of tumor cells. In one study, mouse mammary adenocarcinoma cells, treated with 5-azacytidine and selected for elevated levels of class I MHC expression did not display altered tumorigenicity compared to the parent line (52).

The immune response to tumor cells can be stimulated by systemic administration of IL-2 (53), or IL-2 with LAK cells (54,55). Clinical trials using tumor infiltrating lymphocytes are also in progress (17). Recently, several studies have examined the tumor suppressive effect of lymphokine production by genetically altered tumor cells. The introduction of tumor cells transfected with an IL-2 expression vector into syngeneic mice stimulated an MHC class I restricted cytolytic T lymphocyte response which protected against subsequent rechallenge with the parental tumor cell line (6). Expression of IL-4 by plasmacytoma or mammary adenocarcinoma cells induced a potent anti-tumor effect mediated by infiltration of eosinophils and macrophages (56). These studies demonstrate that cytokines, expressed at high local concentrations, are effective anti-tumor agents.

We have previously proposed an alternative approach to stimulate an anti-tumor response, through the introduction of an allogeneic class I MHC gene into established human tumors. The antigenicity of tumor cells has been altered previously by the expression of viral antigens through infection of tumor cells (57–61), or expression of allogeneic antigens introduced by somatic cell hybridization (62,63). Allogeneic class I MHC genes have been introduced into tumor cells by transfection and subsequent selection *in vitro*. These experiments have produced some conflicting results. In one case, transfection of an allogeneic class I MHC gene (H-2L^d) into an H-2^b tumor resulted in immunologic rejection of the transduced cells and also produced transplantation resistance against the parent tumor cells (64). In another instance, transfection of H-2^b melanoma cells with the H-2D^d gene did not lead to rejection (65), however increased differential expression of H-2D products relative to H-2K may have affected the metastatic potential and immunogenicity of tumor cells (66). The effects of allogeneic H-2K gene expression in tumor cells was examined in another study (67). Several subclones which were selected *in vitro* and expressed an allogeneic gene were rejected in mice syngeneic for the parental tumor line, however, other subclones did not differ from the parental, untransduced line in generating tumors. This finding suggests that clone-to-clone variation in *in vivo* growth and tumorigenic capacity may result in other modifications of cells, caused by transfection or the subcloning procedure, which affects their tumorigenicity. These types of clonal differences would likely be minimized by transducing a population of cells directly *in vivo*.

Because the H-2K class I MHC antigen is strongly expressed on most tissues and can mediate an allogeneic rejection response, we chose it in our animal model studies designed to enhance the immunogenicity of tumors *in vivo*. These studies extended previous efforts to modify tumor cells by developing a system for the direct introduction of genes into tumors by *in vivo* infection using retroviral vectors or by DNA/liposome mediated transfection. This technology can also be used to deliver specific recombinant cytokines into the tumor microcirculation and to understand the immunologic basis for tumor rejection *in vivo*.

2.0 Objectives

The immune system can provide protection against cancer and may play an important role as an adjuvant treatment for malignancy. Lymphokine activated killer cells (LAK) and tumor infiltrating lymphocytes (TIL) can lyse neoplastic cells and produce partial or complete tumor rejection. Expression of cytokine genes in malignant cells has also enhanced tumor regression. Because current strategies to stimulate an immune response against tumor cells often fail to eradicate tumors, an important goal of immunotherapy is to improve upon current techniques and understand the mechanisms of immune recognition.

In this study, we attempt to improve upon our previous studies to enhance the immune response against tumors. We have utilized direct gene transfer into tumor cells *in vivo* as a method to simplify the delivery of genes *in vivo*. Traditionally, gene transfer techniques have focused on modification of tumor cells *in vitro*, followed by transfer of modified cells. Such approaches subject these cells to selection and different growth conditions from those which act *in vivo*. Because they also require that cell lines be established for each malignancy, adaptability to human disease is more difficult and requires more time.

We have previously described a model for the immunotherapy of malignancy using a gene encoding a transplantation antigen, an allogeneic class I major histocompatibility complex (MHC) antigen, introduced into human tumors *in vivo* by DNA/liposome transfection (1,2). Expression of allogeneic MHC antigens on tumor cells stimulates immunity against both the allogeneic MHC gene on transduced cells as well as previously unrecognized antigens in unmodified tumor cells (5). The introduction of an allogeneic MHC gene directly into tumors *in vivo* has induced partial tumor regressions, as well as the specific cytotoxic T cell response to other antigens. In a recent trial in humans, we observed no toxicity of this form of treatment. We have continued to optimize this gene delivery approach, and now propose four improvements on the previous protocol which could improve its ultimate efficacy. These modifications include 1) the use of another cationic lipid formulation, DMRIE/DOPE, 2) optimization of vector expression and inclusion of a cytokine gene, 3) catheter-mediated gene delivery, and 4) application to different tumor types.

Because this approach employs direct gene transfer *in vivo*, it can be applied easily in a clinical setting to spontaneously arising tumors, alone or in combination with cytokines or other adjuvant treatments, including adoptive lymphocyte transfer, to augment tumor immunity. In this study, we propose another phase I clinical trial almost identical to our previous protocol which will evaluate the safety and appropriate dosage for these new modifications. Recombinant gene expression *in vivo* will be documented, and the specificity and mechanism of the immune response will be characterized. Escalating treatment regimens will be used and tumor growth evaluated. These studies will define the safety of the modifications of this approach to the immunotherapy of malignancy and may provide therapeutic effects for patients. Adaptations of this approach might also prove useful in the treatment of other human diseases. Specifically, we plan:

1) To establish a safe and effective dose to introduce either a plasmid vector encoding recombinant HLA-B7 with β-2 microglobulin. Potentially, a plasmid vector encoding re-

combinant HLA-B7 and a cytokine gene could be incorporated in the future.

2) To confirm expression of these genes introduced directly into tumor cells *in vivo*.
3) To analyze the specificity of the immune response against this antigen *in vivo* by analyzing cellular and humoral immunity.

3.0 Selection of Patients

Patients will be carefully selected based on consideration of their past medical history and present status. The referring physician, the attending physician, and surgical oncology or the relevant clinical department, the patient, and family members will make a joint decision regarding the appropriate treatment with conventional therapy. If surgery, chemotherapy, or radiation are deemed unlikely to provide further benefit to the patient, the opportunity to pursue this experimental protocol will be offered to the patient. The following criteria will be used to select appropriate patients for study:

3.1 Criteria for Patient Eligibility

3.1.1 Patients must be HLA-B7 negative.
3.1.2 Patients with cutaneous tumor nodules will be preferred in patients undergoing intratumor injection.
3.1.3 The patient's disease will be unresponsive to standard modes of treatment.
3.1.4 Patient must be greater than 18 years of age.
3.1.5 The patient must not have antibodies to the human immunodeficiency virus.
3.1.6 The patient must be able to provide informed consent.
3.1.7 Estimated life expectancy >2 months and ECOG performance status of 0–1.

3.2 Criteria for Patient Ineligibility

3.2.1 Patients with active autoimmune disease or infection.
3.2.2 Patients who have a positive antibody to HIV.
3.2.3 Patients with active hepatitis, chronic or acute.
3.2.4 Patients with diabetes mellitus who are not controlled by medical treatment.
3.2.5 Psychiatric illness which makes compliance to the clinical protocol unmanageable.
3.2.6 Patients with unstable angina or complications of cardiovascular disease which would contraindicate catheterization, if relevant.
3.2.7 Patients on high dose glucocorticosteroids.
3.2.8 Patients who undergo an alternative mode of anticancer treatment will not be accepted within 4 weeks of the last treatment.

4.0 Clinical Evaluation Prior to Treatment

As described above, patients will be selected based on their past medical history and clinical course. Patients must have histologically confirmed metastatic melanoma which is unresponsive to standard curative or palliative measures. Patients with expected survival times of 1 year or less will be chosen. The patients must meet the standard inclusion and exclusion criteria described above. In addition, the chosen patients must show no signs of active systemic infection or major cardiovascular or respiratory disease. The following laboratory values will be used as guidelines as enrollment:

1. The bilirubin should be less than 2 mg/dl. The platelet count should be greater than 100,000/mm^3. The white blood cell count should exceed 3,000/mm^3. The creatinine should be less than 2.0 mg/dl.
2. HLA typing must indicate that the patient is HLA-B7 negative.
3. HIV antibody titers will be measured.

5.0 Stratification and Randomization

Not relevant.

6.0 Nature of Procedures or Therapeutic Agents

6.1 Study Design

Patients will be admitted to the Clinical Research Center at The University of Michigan Medical Center after the relevant eligibility criteria have been met. The pre-treatment evaluation will be performed as described in Section 9. Patients undergoing the direct gene transfer protocol will have serum samples removed for analysis as described in Section 9. The tumor nodule to be injected will be identified and its borders measured prior to injection. A needle biopsy will be performed to confirm the diagnosis and to analyze as a pretreatment sample. Tissue will be stored as frozen sections for further immunohistochemical analysis and PCR. In addition, this nodule and other control (untreated) nodules will be imaged by CT immediately prior to the procedure, and its size quantitated. The skin overlying the tumor nodule will be sterilized and anesthetized using 0.01% lidocaine. For gene transfer, a 22-gauge needle will be used to inject the DNA liposome complex which will be prepared as follows: 10 minutes prior to delivery, 0.1 ml of plasmid DNA (0.05–50 mg/ml) in lactated Ringer's solution is added to 0.1 ml of DMRIE/DOPE liposome solution (0.15–15 μM). Each component will be stored separately in sterile vials and certified as acceptable by the FDA. The solution is left at room temperature for 5–10 minutes and 0.8 ml of sterile lactated-Ringer's is added to the liposome DNA solution. The optimal composition of the DNA/liposome complex has been established for each batch by titration of DNA concentration and liposome concentration independently on human melanoma and renal cell carcinoma *in vitro*, and confirmed by direct injection into melanoma or other tumors *in vivo* prior to use. Each component, the liposome preparation and the DNA, will be tested for contaminants and toxicity and used according to previously established guidelines from the FDA. The liposome solution and DNA will be aliquoted in individual sterile vials and mixed under sterile conditions as described above.

For direct injections of the HLA-B7 plasmids, escalating doses will be studied in this phase I study. Four groups (3 patients each) will be studied sequentially with at least 1 month of observation prior to evaluation of the next group. Patients in each group will receive intratumor injections. Group I will receive 3 injections of 0.1 ml within the same nodule (3 μg of DNA + 9.0 nM DMRIE/DOPE). Group II will receive the same treatment with a 10-fold higher concentration of DNA liposome complex. Group III will receive 100-fold higher dose, and Group IV will receive 1000 $\times$ higher amount. We are currently evaluating whether pre-treatment with low dose cytoxan can improve the anti-tumor response by eliminating suppressive T cells. If this approach is helpful, it will be included as part of the protocol.

For catheter-based gene delivery, the same dose escalation will be used, except a single 0.6 ml injection into the end artery which perfused an isolated nodule will be used with an occlusion balloon catheter. In murine and porcine models, the highest treatment exceeded these proposed doses by 100-fold and are well-tolerated. Doses will be repeated within each subject for whom the toxicity treatment is $\leq$ grade II. Dose escalation will begin if three patients show toxicities < grade III from the treatment. If one patient displays toxicity > grade II, the treatment will be repeated on three additional patients. If two patients develop toxicity > grade II, the dosage will be reduced. The maximal tolerated dose will be defined as the dose at which two or more patients out of six develop grade III or IV toxicity. The treatment dose will be established at one level below the maximum tolerated dose. Once the treatment dose is defined, an additional four to six patients will be entered at that dose to ascertain the safety of this dose for wider application.

Prior to the injection with the needle in place, gentle aspiration will be applied to the syringe to ensure that no material is injected intravenously. Immediately after the injection procedure, a blood sample will be obtained to check serum enzymes, chemistries, and blood counts, and to analyze for the presence of plasmid DNA in the peripheral blood by PCR. The patients will be observed in the Clinical Research Center for an additional 48 hours, and another blood collection performed as described in Section 9. If there are no complications, the patient will be discharged after 48 hours. Should any abnormalities appear, the patient would be kept for further observation.

6.2 Confirmation of Gene Transfer and Expression

Needle biopsy of the injected nodule will be performed after administration of local anesthesia prior to injection and subsequently to treatment (preferably at day 3). A portion of this tissue will be processed to obtain DNA for PCR analysis. The remaining tissue will be processed for pathologic analysis and immunohistochemical and/or immunofluorescent staining. If sufficient material can be obtained, RNA PCR analysis will also be performed. For internal organs, CT or ultrasound guided thin needle biopsies will be obtained when possible.

6.3 Analysis of Immune Response

Evidence of gene transfer can also be obtained indirectly by examination of the specific immune response to HLA-B7. The analysis will be performed as follows: two weeks prior to the initial treatment, a blood sample will be obtained to derive lymphocytes which will be immortalized using the Epstein-Barr virus. An aliquot of these cells will be further infected with an amphotropic HLA-B7 retroviral vector, and expression will be confirmed on the cell surface. These cells will subsequently be used in the laboratory as target cells for the cytolytic T cell assay. At no time will these cells be brought into the same building where the patient is being treated. These cells and laboratory experiments will be performed in MSRB II, Room 3560, whereas the patient's clinical treatment will be taking place in the Clinical Research Center of The University of Michigan Medical Center Hospital.

6.4 Repeated Treatment

If no adverse side effects of the treatment are observed, repeated injections will be considered at two-week intervals. Doses identical to the initial treatment regimen will be repeated with similar protocols and observation as described in Section 6.1.

6.5 Postmortem Analysis

The life expectancy of patients who enter this protocol will be limited, in general, less than six months. Important information can be obtained by analysis of tissue postmortem, including presence of DNA in germ line tissue, distribution of DNA and immune function, and potential toxicity. To maximize the information derived from these studies, every effort will be made to perform postmortem analyses. Consent for an autopsy will not be required for entry, but patients and their families will be informed of this aspect of the study and its potential contribution to medical knowledge.

7.0 Schema/Duration (see Table 1)

8.0 Dose of Therapeutic Modifications

Patients can receive medications to control any side effects of treatment. Such agents would include acetaminophen (650 mg. q. 4 hrs.), indomethacin (50–75 mg. q. 6 hrs.), or antihistamines as required. Glucocorticoids will not be used in these patients; or if such medication is required, the patient will be removed from the protocol.

9.0 Study Parameters (revised)

9.1 Pre-treatment (see Table 2)

The following studies will be performed as summarized in Table 2.

a. Complete physical examination noting in detail the exact size and location of any lesions that exist. The tumor lesion to be treated will be imaged and its dimensions quantitated prior to treatment.

TABLE 1. SCHEMA OF CLINICAL PROTOCOL FOR (A) TUMOR INJECTION OR (B) CATHETER DELIVERY

A. TUMOR INJECTION—CUTANEOUS MELANOMAS

Group	*No. of injections per treatment*	*Volume of injection (ml)*	*Times of repeated treatment*	*Total number of treatments*	*Dose DNA*	*Liposome*
I	3	0.2 × 3	2 wk.	3	0.003 mg	0.009 μM
II	3	0.2 × 3	2 wk.	3	0.03 mg	0.09 μM
III	3	0.2 × 3	2 wk.	3	0.3 mg	0.9 μM
IV	3	0.2 × 3	2 wk.	3	3 mg	9.0 μM

B. CATHETER DELIVERY—MELANOMA OR OTHER MALIGNANCIES

Group	*No. of injections per treatment*	*Volume of injection (ml)*	*Times of repeated treatment*	*Total number of treatments*	*Dose DNA*	*Liposome*
I	1	0.6	2 wk.	3	0.003 mg	0.009 μM
II	1	0.6	2 wk.	3	0.03 mg	0.09 μM
III	1	0.6	2 wk.	3	0.3 mg	0.9 μM
IV	1	0.6	2 wk.	3	3 mg	9.0 μM

b. Complete chemistry survey including electrolytes, liver function tests, calcium, magnesium, creatinine, BUN, CPK, pancreatic enzymes.
c. CBC, differential count, PT, PTT, platelet count.
d. Urine analysis and culture.
e. Hepatitis screen.
f. HIV titer.
g. Pregnancy test for women.
h. Chest x-ray.
i. Electrocardiogram.
j. Baseline x-rays and nuclear medicine scans to evaluate the status of disease.
k. CT scan or MTI scan of brain.
l. 45 ml of clotted blood for serum storage and 45 ml of anti-coagulated blood for mononuclear cell cryopreservation.
m. Biopsy of tumor, if possible with minimal morbidity.

9.2 Treatment

Patients will be analyzed with routine blood and chemistry analysis. A chest x-ray will be performed each month for six months and every six months thereafter or more often if needed. During the gene transfer protocol, patients will be monitored closely in the Clinical Research Center. Vital signs will be measured every 15 minutes prior to, during, and after the injection for at least two hours or until the patient is stable. If the systolic blood pressure drops below 80 mm/Hg, the injection will be terminated immediately.

9.3 Post-treatment

Cutaneous lesions will be evaluated by physical examination, biopsy if feasible, and appropriate imaging scans prior to injection, monthly for 2 months or as needed to evaluate response to treatment. Serum will be analyzed by PCR for the presence of plasmid, and antibody to HLA-B7 will be evaluated. PBL's will be isolated and analyzed for their ability to lyse HLA-B7 modified autologous EBV-transformed lines. If ANA becomes positive or other signs of autoimmunity appear, evidence of more specific autoantibodies, e.g., anti-Rho, Smith, etc., will be tested and rheumatology consultation will be sought.

9.4 Potential Side Effects and Reporting of Adverse Reactions

Adverse Drug Reactions will be reported to the Drug Information Service, University of Michigan Medical Center, phone 313-936-8200 or 313-936-8251 (available 24 hours), and include all life-threatening events (Grade 4) which may be due to drug administration, all fatal events, or the first occurrence of any *previously unknown* clinical event (regardless of Grade). A written report is to follow within 10 working days to:

Investigational Drug Branch
P.O. Box 30012
Bethesda, Maryland 20824
Phone: 301-230-2330
Fax: 301-230-0159

Data will be submitted to the IRB yearly or upon request. All adverse reactions will be reported to the IRB, even if there is only a suspicion of a drug effect. All side effects will be graded using the standard toxicity sheet described in Section 14, Common Toxicity Criteria.

9.4.1 Potential Risks of Gene Transfer In Vivo

Insertional mutagenesis. The possibility of causing malignancy in cells secondary to the random insertion of the DNA in the genome exists, though this risk is considered low. There is a remote chance that the vector could replicate in the host. Neither finding has been seen in animal studies (~200 mice, rabbits, and pigs); nonetheless, the proposed protocol provides for extensive testing of tumor tissue and blood after injection into the patient. PCR will be performed to monitor for this unlikely event.

The use of aminoglycoside antibiotics. The neomycin resistance gene, which encodes neomycin phosphotransferase (NPT), phosphorylates the 3′ hydroxyl group of the aminohex-

TABLE 2. TREATMENT PARAMETERS

		During Treatment					
		Day				*Week*	
	Pre-Study	*1*	*3*	*14*	*28*	*6*	*8*
Physical exam	X	X		X	X	X	X
History	X						
Performance Status	X						X
Tumor Staging and/or	X			X	X	X	X
Nodule Imaging							
Chemistry Survey[1]	X	X	X	X	X	X	X
Vital Signs	X	X	X	X	X	X	X
Weight	X	X	X	X	X	X	X
CBC, Diff, Platelet	X	X	X	X	X	X	X
PT, PTT	X	X		X	X		X
EKG	X		X				
CPK + isoenzymes	X	X		X	X	X	X
CXR	X				X		X
U/A and Culture	X						
Hb_sAg, HIV	X						
Brain CT or MRI	X						
Assess for adverse events status	X	X	X	X	X	X	X
PCR or PBL and serum to	X	*X	X	X	X	X	X
detect HLA-B7 gene							
Tumor biopsy	**X			X	X	(X)	(X)
Assay for HLA-B7 cytotoxic	X			X	X		X
T cells							
Assay for HLA-B7 antibody	X			X	X		X
Pregnancy test (if relevant)	X						
Anti-DNA antibodies	X				X		X
ds DNA							
ss DNA							
CT scan of brain (if relevant)	X				X		X
CT of chest & abdomen	X					X	(X)
Quantitate size of lesion	X			X	X		X
ANA, ESR, CH50, CRP	X				X		X

* An additional sample will be taken several minutes after injection to examine for the presence of plasmid DNA.
** To be performed on nodules prior to treatment. Nodules will be between 0.25 and 4 cm in size.
[1] Routine laboratory tests include uric acid, calcium, phosphate, SGOT, SGPT, alkaline phosphatase, LDH, bilirubin, BUN, creatinine, total protein, glucose, amylase, lipase.

ose I of neomycin and its analogues, inactivating the antibiotic. While amikacin may be inactivated by this enzyme, gentamicin and tobramycin do not contain an hydroxyl at the 3′ position and are not inactivated. Therefore, introduction of the NeoR gene would not exclude the use of aminoglycosides or any other conventional antibiotic that may be needed in the clinical management of these patients.

Although risks to the patient exist in this study, they would appear to be minimal, and the escalating dose nature of this study should minimize the risks to any individual patient. Because these patients have limited life expectancy from their advanced cancer, these risks are thought to be justified, considering the potential therapeutic benefit.

10.0 Off-Study Criteria

Patients will be removed from the study should any grade 3 or 4 toxicity develop which is not easily corrected. The toxicity sheet is included in Section 14.0, Common Toxicity Criteria).

If two treatment-related deaths should occur, this protocol will be terminated.

11.0 Evaluation of Results

11.1 Criteria for Response

In this phase I study, the protocol will be considered successful if recombinant gene expression is achieved and an appropriate dosage established for effective gene transfer without toxicity. Confirmation of recombinant gene expression is described below. Additional evidence of successful gene transfer may also be obtained from immunologic analysis. A new CTL or antibody response to HLA-B7 will indicate successful gene expression and is described in Section 11.3. The ability to generate anti-tumor CTL will also be evaluated. Evidence for tumor regression will also be obtained. *Complete tumor response* is defined as the disappearance of all clinical evidence of disease for at least four weeks. *Partial tumor response* is de-

fined as 50% or greater decrease of the sum of the products of perpendicular diameters of lesions lasting at least four weeks with no increase in existing lesions or appearance of new lesions. The response will also be considered positive for gene transfer if cytolytic T cell activity for HLA-B7 is obtained after this injection. Any patient having less than a partial response is considered to be non-responsive to treatment. Tumor dimensions will be assessed by imaging as detailed in Section 9.

11.2 Confirmation of Recombinant Gene Expression

Several independent techniques will be used to evaluate the presence and expression of the recombinant gene *in vivo*. We have used several monoclonal antibodies to HLA-B7 to detect the recombinant gene product *in vivo* by immunohistochemistry. Fluorescence staining of freshly dispersed cells will also be evaluated. The presence of plasmid DNA will be confirmed by PCR of DNA from tumor tissue, peripheral blood lymphocytes, or in autopsy specimen tissue. If sufficient tissue is available, RNA will be isolated and examined for the presence of HLA-B7 mRNA by PCR or S1 nuclease analysis.

11.3 Analysis of Immune Response

Direct gene transfer and expression of the HLA-B7 gene may sensitize the patient to HLA-B7 and lead to the generation of an immune response to this antigen. Limiting dilution analysis (LDA) will be utilized to evaluate alterations in the frequency of helper and cytolytic T cells for HLA-B7 in the peripheral blood following direct gene transfer. Peripheral blood lymphocytes will be isolated and cryopreserved prior to, and at 4-week intervals, following the initial direct gene transfer. At the completion of treatment, samples of PBL from each time point will be simultaneously evaluated for responsiveness to HLA-B7 by culturing PBL, under LDA conditions, with autologous EBV-B cells transduced with the HLA-B7 gene. Antigen specific elaboration of IL-2 or generation of CTL to HLA-B7 positive target cells will be the indices evaluated in these studies. The presence of antibody will be evaluated by FACS analysis of a matched pair of HLA-B7^{+} or HLA-B7^{-} cell lines. In some instances, lymphocytes will be isolated directly from the tumor, expanded in tissue culture, and analyzed for cytolytic function. Tumor biopsies at 7–14 days after treatment will be analyzed by immunohistochemistry. If possible, we will attempt to expand draining lymph node T cells or TIL cells to test their cytolytic function. When possible, we will derive autologous cell lines to be used as targets in a ^{51}Cr release assay. Finally, every attempt will be made to excise tumor tissue prior to treatment for diagnosis, immunohistochemistry, and cryopreservation and to evaluate delayed type hypersensitivity reactions to the tumor before and after treatment.

11.4 Toxicity

Toxic side effects of treatment will be analyzed and classified by the common toxicity criteria (Section 13).

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INJECTION INFORMED CONSENT

University of Michigan Medical School
Department of Internal Medicine
and the Clinical Research Center

CONSENT FORM

(To be read by the Patient and explained to the Patient by his or her Physician).

PROTOCOL: Immunotherapy for Cancer by Direct Gene Transfer into Tumors

PRINCIPAL INVESTIGATOR: Gary J. Nabel, M.D., Ph.D.

CO-PRINCIPAL INVESTIGATORS:
Alfred E. Chang, M.D.
Elizabeth G. Nabel, M.D.
Gregory E. Plautz, M.D.
William Ensminger, M.D.

PROTOCOL NUMBER IRB 93-129
PATIENT NAME: ______________________
DATE: ______________________
HOSPITAL NO.: ______________________

INTRODUCTION

We invite you to participate in a research study at the University of Michigan Medical Center. Several general principles apply to all who take part in any experimental studies:

(1) Your participation in this study is voluntary
(2) You may not personally benefit from this study, but knowledge may be gained from it that will benefit others
(3) You may withdraw from the study at any time for any reason without jeopardizing your further care.

The nature of the study, the risks, inconveniences, discomforts, and other information are discussed in the following sections. Please do not hesitate to discuss any questions you have about this study with the physicians who explain it to you.

DESCRIPTION OF TREATMENT OR PROCEDURE TO BE UNDERTAKEN

As you are aware, you have cancer which cannot be cured by medicine, surgery, or radiation. In this study, treatments will be offered that may help to fight this disease in future patients. Because the treatment is experimental, you may not derive any direct benefit from it. The purpose of this study is to determine a safe and effective dose of a new treatment which will attempt to induce tumor regression. Because this is a new, experimental treatment, we will also be observing you to determine the side effects of the therapy. We will also monitor the effects of this treatment on the growth of your tumor.

By using techniques in the laboratory, it is now possible to prepare large amounts of human DNA or genetic material in bacteria. This DNA will be mixed with fat bodies called liposomes, and we plan to transport the mixture into your tumor by needle injection. Once introduced into the tumor, the DNA produces proteins which stimulate tissue rejection. One protein—known as HLA-B7—causes the cells which will contain it to be recognized as "foreign enemy" by your immune system and, in some cases, a second protein, called interleukin-2, will be made. This protein also causes activation of your immune system. The purpose of our study is to determine whether this treatment will induce the cells of your immune system, known as lymphocytes, to attack and kill your tumor. This type of therapy which stimulates your lymphocytes is called immunotherapy.

Alternative Therapies

There are no known cures for patients with your disease. Other alternative treatments available to you can control local symptoms. These include the delivery of x-ray treatment to sites of local disease to control pain, medication to control pain, and medical, surgical, or radiation treatment of any reversible complications. Experimental drugs are being evaluated at other centers to which you can be referred. Other experimental treatments are under investigation which attempt to stimulate your immune system to reject your tumor, and you can be referred to physicians who are conducting such trials. In contrast to this treatment, other protocols usually require removal of tumor cells or blood cells, which are taken to the laboratory for genetic manipulation, and subsequently returned to you by injection. In some cases, proteins are injected which can stimulate the immune system. You also have the option to receive no treatment at this time.

Procedures

Before receiving this treatment, you will receive many tests to see if you qualify for this study. These tests will be made either as an inpatient or an outpatient. These tests include: 1) blood tests 2) x-rays of the brain, chest and abdomen and 3) a blood test for the antibodies to Human Immunodeficiency Virus (HIV), which causes AIDS. If you have antibodies to HIV, you may not participate in this study.

If you qualify for this study, we will inject a solution containing the DNA/liposome complex directly into a tumor nodule. The injections will be made under sterile conditions after providing a local anesthetic (xylocaine), and multiple areas within a single nodule will be injected up to 5 times. The time of treatment is usually ~30 minutes. The treatment will be repeated every 2 weeks for a total of three. Blood samples (between 1–10 tubes) will be obtained daily at first, then weekly for the 1st month, and biweekly for the next month. A CT scan will be performed before initiation of treatment and at the end of the 2-month study period. Your blood lymphocytes will be tested for their ability to respond to the HLA-B7 antigen. We will also examine your blood for evidence of toxicity from this treatment.

At different times in the protocol, tumor biopsies will be performed. This procedure involves the injection of a local anesthetic (xylocaine) under sterile conditions, followed by insertion of a needle into the tumor nodule and withdrawal of a sample of the tumor. This procedure will be performed prior to treatment and at intervals of 2 weeks up to 4 times.

RISKS AND SIDE EFFECTS

There are potential side effects and risks to this procedure. First, you may experience mild discomfort from needle injections or tumor biopsies. You may have mild discomfort and bleeding from the tumor biopsy. You will be given a local anesthetic to minimize the discomfort. Second, even though the DNA inserted into your tumor is considered harmless to you, events could occur within normal cells that allow them to become cancerous. Laboratory studies suggest that this possibility is unlikely. However, this is a new procedure and we do not know whether cells could become abnormal after long periods of time. In animal studies, we have not observed the development of cancer cells in any animals tested. Third, the inserted DNA will contain a gene that inactivates certain antibiotics in bacteria. This protein is not likely to be made in humans, and many other antibiotics that are not inactivated will be available and effective in treating any potential bacterial infections. Finally, it is possible that expression of this gene could induce unanticipated changes in your immune system that could lead to autoimmune diseases, such as arthritis. Studies in animal models suggest that this possibility is unlikely.

We emphasize that this procedure, called direct gene transfer, has never been used before in human patients. Because this procedure is new, it is possible that despite our extensive efforts, other unforeseen problems may arise, including the very remote possibility that death may occur.

You will undergo biopsy of tumor and other tissue, if available, on several occasions before and after injection. Blood and tissue specimens will be taken where possible to follow the duration and effects of HLA-B7 expression. If we are successful in this protocol, you will be immunized to the HLA-B7 protein. In the event that you should require an organ transplant, you would not be able to receive an organ from an individual who makes this protein, on average, ~15% of donors.

Follow-Up

After you receive the treatment, you will be discharged from the hospital if you have no other significant medical problems. You will be required to return to the University of Michigan Medical Center for follow-up studies described above for at least 8 weeks after the trial has begun. It is possible that we may ask you to return after this time if additional tests will be needed. Tests used to decide if your tumor has responded to the therapy will be similar to those you had before beginning the therapy. If your disease recurs after treatment in this protocol, you will be eligible for other protocols and will receive treatment as indicated by your disease or referred elsewhere for such treatment. Because this form of therapy is new, unanticipated side effects that may cause your condition to deteriorate could be encountered. You will be closely monitored for such side effects.

Treatment will continue as long as there is sufficient possibility of response to warrant the risks and side effects encountered. Your physicians feel that the risks of your disease are much greater than the risks of the treatment as outlined above. Furthermore, your physicians have considered your individual situation and have concluded that, at this time, no other therapeutic approaches such as surgery, radiation therapy, or other chemotherapeutic treatments are clinically indicated as being more effective. At some later time, should these alternatives be clinically indicated, they will be discussed with you because this study does not preclude their use.

Consent for Autopsy

To fully evaluate the effects and safety of gene transfer, it will be necessary to obtain as much information as possible. In the event or occurrence of your death, evaluation of your organs will be a very valuable method to see the full effects of gene transfer. Please understand that we will therefore make a request for an autopsy from your next-of-kin in the event of your death from any cause, and we would encourage you to discuss this possibility with your family in advance.

Contraception

Because genetic material could circulate to multiple sites in the body, there is a small possibility that it could be unintentionally transmitted to unborn offspring where it could possibly have harmful effects. To avoid this possibility, you will be required to practice contraception for the 2-month duration of this program. To help us learn about the potential for this side effect, male participants will be asked to provide semen specimens for laboratory studies.

Other Pertinent Information

1. Confidentiality. When results of a study such as this are reported in medical journals or at meetings, the identification of those taking part is withheld. Medical records are maintained according to current legal requirements, and are made available for review, as required by the Food and Drug Administration or other authorized users, only under the guidelines established by the Federal Privacy Act. A qualified representative of the National Institutes of Health may inspect patient and study records. This procedure may attract attention from the media. We will make every effort to protect your confidentiality. Because of media interest, however, there is a significant chance that information concerning you and your treatment will appear publicly without your consent.
2. Policy regarding research-related injuries. In the unlikely event of physical injury resulting from research procedures, the University will provide first-aid medical treatment. Treatment of injuries or side effects directly related to this experimental treatment will be provided at no cost to you. Additional medical treatment will be provided in accordance with the determination by the University of its responsibility to provide such treatment. However, the University does not provide compensation to a person who is injured while participating as a subject in research. If you have questions regarding additional medical treatments, you may call the Patient Staff Relations Office (313/763-5456).
3. You will not be paid to take part in this study.
4. Outpatient and inpatient studies will be ordered to determine your eligibility for study and whether you have had a response to the treatment. Clinic visits, laboratory tests, x-rays, and scans will not be free. Items which are not covered by insurance which relate to this research protocol will be covered by research grants. The cost of tests and treatments unrelated to this study will be handled as usual and will depend on whether or not you have insurance and what costs your insurance covers. Unfortunately, insurance coverage cannot be guaranteed for all tests and treatments; however, you may discuss this issue with the hospital financial office or your insurance company before you agree to participate.
5. This consent form does not include consent relating to the risks of any surgical procedures. Any surgical procedures performed will require a separate consent form.

Please understand that you are free to withdraw your consent to participate in this study at any time during treatment or follow-up and seek care from any physician with no loss of benefits or disruption in your care.

The team of physicians and nurses providing care in this study are very experienced. You must realize, however, that unforeseeable or unexplained risks are always possible when investigational therapies are undertaken.

If you have questions pertinent to this research, you should contact Gary J. Nabel, M.D., Ph.D., at 313/747-4798.

If you feel that you have a research-related injury, contact Gary J. Nabel, M.D., Ph.D., at 313/747-4798.

The following numbers are for your use if medical problems develop during treatment:

Office: 313/747/4798
Clinical Research Center: 313/936-8090
Voice Mailbox: 313/764-9121 (after office hours)
Doctor or Nurse: 313/936-6266; Paul Watkins, M.D.
(This is a hospital beeper; ask the paging operator to page #9128. Use the following numbers in case of an Emergency Only).
(Outside of regular office hours if the paging service won't do.)
Home: 313/000-0000; Dr. Gary J. Nabel

Questions on my rights as a patient may be directed to Ann Munro in the Patient/Staff Relations Office at 313/763-5456.

I have fully explained to the patient, ________________, the nature of the treatment program described above and such risks as are involved in its performance.

____
Physician's Signature

I have been fully informed as to the procedures to be followed including those which are investigational, and have been given a description of the attendant discomforts, risk, and benefits to be expected, and the appropriate alternative procedures. I realize that, since my participation is voluntary, I can refuse this treatment without in any way prejudicing my future medical care. In signing this consent form, I agree to this method of treatment, and I understand that I will receive the best supportive care even if not receiving this protocol treatment. I also understand that my doctors can stop my treatment on this protocol if they feel the risks in my case have increased, over time, to exceed the potential benefits to me. I understand, also, that if I have any questions at any time, they will be answered. I have received a copy of this consent form.

I am not and will not become pregnant during this study (female participants). I will use contraception to avoid inducing a pregnancy while receiving treatments on this protocol (male and female participants).

I understand that the University will provide first-aid medical treatment in the unlikely event of physical injury resulting from research procedures. Treatment of injuries or side effects directly related to the experimental treatment will be provided at no cost to me. Additional medical treatment will be provided in accordance with the University's determination of its responsibility to do so. The University does not, however, provide compensation to a person who is injured while participating as a subject in research.

I have not engaged in any other research projects within the past six (6) months. [ ].

Within the past six (6) months, I have been involved in a study by Dr. ________________.

I have [ ] have not [ ] been under the care of a physician within the past twelve (12) months.

____
Signature of Patient

Date

Witness

CATHETER INFORMED CONSENT

University of Michigan Medical School
Department of Internal Medicine
and the Clinical Research Center

CONSENT FORM

(To be read by the Patient and explained to the Patient by his or her Physician).

PROTOCOL: Immunotherapy for Cancer by Direct Gene Transfer into Tumors

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Gregory E. Plautz, M.D.
William Ensminger, M.D.

PROTOCOL NUMBER IRB 93-129
PATIENT NAME: ____________________
DATE: ____________________
HOSPITAL NO.: ____________________

INTRODUCTION

We invite you to participate in a research study at the University of Michigan Medical Center. Several general principles apply to all who take part in any experimental studies:

(1) Your participation in this study is voluntary
(2) You may not personally benefit from this study, but knowledge may be gained from it that will benefit others
(3) You may withdraw from the study at any time for any reason without jeopardizing your further care.

The nature of the study, the risks, inconveniences, discomforts, and other information are discussed in the following sections. Please do not hesitate to discuss any questions you have about this study with the physicians who explain it to you.

DESCRIPTION OF TREATMENT OR PROCEDURE TO BE UNDERTAKEN

As you are aware, you have cancer which cannot be cured by medicine, surgery, or radiation. In this study, treatments will be offered that may help to fight this disease in future patients. Because the treatment is experimental, you may not derive any direct benefit from it. The purpose of this study is to determine a safe and effective dose of a new treatment which will attempt to induce tumor regression. Because this is a new, experimental treatment, we will also be observing you to determine the side effects of the therapy. We will also monitor the effects of this treatment on the growth of your tumor.

By using techniques in the laboratory, it is now possible to prepare large amounts of human DNA or genetic material in bacteria. This DNA will be mixed with fat bodies called liposomes, and we plan to transport the mixture into your tumor by needle injection. Once introduced into the tumor, the DNA produces proteins which stimulate tissue rejection. One protein—known as HLA-B7—causes the cells which will contain it to be recognized as "foreign enemy" by your immune system and, in some cases, a second protein, called interleukin-2, will be made. This protein also causes activation of your immune system. The purpose of our study is to determine whether this treatment will induce the cells of your immune system, known as lymphocytes, to attack and kill your tumor. This type of therapy which stimulates your lymphocytes is called immunotherapy.

Alternative Therapies

There are no known cures for patients with your disease. Other alternative treatments available to you can control local symptoms. These include the delivery of x-ray treatment to sites of local disease to control pain, medication to control pain, and medical, surgical, or radiation treatment of any reversible complications. Experimental drugs are being evaluated at other centers to which you can be referred. Other experimental treatments are under investigation which attempt to stimulate your immune system to reject your tumor, and you can be referred to physicians who are conducting such trials. In contrast to this treatment, other protocols usually require removal of tumor cells or blood cells, which are taken to the laboratory for genetic manipulation, and subsequently returned to you by injection. In some cases, proteins are injected which can stimulate the immune system. You also have the option to receive no treatment at this time.

Procedures

We would like to deliver this treatment through a tube or catheter in your blood vessels. In this procedure, a small tube (catheter) is introduced into one or several of your blood vessels. Through this tube, a solution containing iodine (contrast medium) will be injected, which will enable us to see the blood vessels of the tumor on x-rays. After the vessel which supplies a tumor nodule in your lungs is identified, we will inject the genetic material complexed to fat particles that will allow HLA-B7 protein to be made in an attempt to stimulate your body to attack this nodule. The tube is introduced into the blood vessels, either in your arm or your groin, by means of minor surgery under local anesthesia. The entire procedure will take 1–3 hours to perform, of which the gene transfer will take ~15 minutes. It will be repeated at 2-week intervals for a total of three times. Although the possibility of clotting the vessel used is small, it does happen occasionally. In addition, it is possible that an artery or arteries feeding an organ could also be clotted. In either of these circumstances, it may be necessary to perform surgery to remove the clot or to treat you with certain medications which may dissolve the clot. We are sure you realize that although the risk is very small, clotting the blood supply to an organ can result in the loss of that organ. The overall serious

complication rate is about 1 in 500 patients. Very rarely, a serious complication can result in death.

During the procedure, it is possible that the contrast medium might result in an adverse reaction, causing hives, shortness of breath, extremely low blood pressure, and very rarely, temporary or permanent paralysis. Also, if the procedure involves the heart or lungs, an abnormal heart rhythm may occur, requiring treatment.

The usual complications, which we would consider minor but which nevertheless can be distressing to the patient, are accumulation of blood in the tissues where the catheter has been introduced (hematoma), or a small bulge of the artery at the site where it was entered by the catheter. If you desire, these will be discussed with you.

Unfortunately, this information may have alarmed you, but we believe it to be in your best interest to understand what is involved. Any questions you have about the above material can be directed to Dr. Nabel, or the physician or the radiologist performing the angiogram. Before proceeding, we request your authorization as indicated below.

Before receiving this treatment, you will receive many tests to see if you qualify for this study. These tests will be made either as an inpatient or an outpatient. These tests include: 1) blood tests 2) x-rays of the brain, chest, and abdomen and 3) a blood test for the antibodies to Human Immunodeficiency Virus (HIV), which causes AIDS. If you have antibodies to HIV, you may not participate in this study.

If you qualify for this study, we will inject a solution containing the DNA/liposome complex directly into a tumor nodule. The injections will be made under sterile conditions after providing a local anesthetic (xylocaine), and multiple areas within a single nodule will be injected up to 10 times. Blood samples (between 1–10 tubes) will be obtained daily at first, then weekly for the 1st month, and biweekly for the next month. Your blood lymphocytes will be tested for their ability to respond to the HLA-B7 antigen. We will also examine your blood for evidence of toxicity from this treatment.

At different times in the protocol, tumor biopsies will be performed. This procedure involves the injection of a local anesthetic (xylocaine) under sterile conditions, followed by insertion of a needle into the tumor nodule and withdrawal of a sample of the tumor. This procedure will be performed prior to treatment and at intervals of 2 weeks up to 4 times.

RISKS AND SIDE EFFECTS

There are potential side effects and risks to this procedure. First, you may experience mild discomfort from needle injections or tumor biopsies. You may have mild discomfort and bleeding from the tumor biopsy. You will be given a local anesthetic to minimize the discomfort. Second, even though the DNA inserted into your tumor is considered harmless to you, events could occur within normal cells that allow them to become cancerous. Laboratory studies suggest that this possibility is unlikely. However, this is a new procedure and we do not know whether cells could become abnormal after long periods of time. In animal studies, we have not observed the development of cancer cells in any animals tested. Third, the inserted DNA will contain a gene that inactivates certain antibiotics in bacteria. This protein is not likely to be made in humans, and many other antibiotics that are not inactivated will be available and effective in treating any potential bacterial infections. Finally, it is possible that expression of this gene could induce unanticipated changes in your immune system that could lead to autoimmune diseases, such as arthritis. Studies in animal models suggest that this possibility is unlikely.

We emphasize that this procedure, called direct gene transfer, has never been used before in human patients. Because this procedure is new, it is possible that despite our extensive efforts, other unforeseen problems may arise, including the very remote possibility that death may occur.

You will undergo biopsy of tumor and other tissue, if available, on several occasions before and after injection. Blood and tissue specimens will be taken where possible to follow the duration and effects of HLA-B7 expression. If we are successful in this protocol, you will be immunized to the HLA-B7 protein. In the event that you should require an organ transplant, you would not be able to receive an organ from an individual who makes this protein, on average, ~15% of donors. During the catheter procedure, there are several risks, including the potential for clot formation or a reaction to intravenous contrast. These risks are described more fully in the previous section entitled "Procedures."

Follow-Up

After you receive the treatment, you will be discharged from the hospital if you have no other significant medical problems. You will be required to return to the University of Michigan Medical Center for follow-up studies described above for at least 8 weeks after the trial has begun. It is possible that we may ask you to return after this time if additional tests will be needed. Tests used to decide if your tumor has responded to the therapy will be similar to those you had before beginning the therapy. If your disease recurs after treatment in this protocol, you will be eligible for other protocols and will receive treatment as indicated by your disease or referred elsewhere for such treatment. Because this form of therapy is new, unanticipated side effects that may cause your condition to deteriorate could be encountered. You will be closely monitored for such side effects.

Treatment will continue as long as there is sufficient possibility of response to warrant the risks and side effects encountered. Your physicians feel that the risks of your disease are much greater than the risks of the treatment as outlined above. Furthermore, your physicians have considered your individual situation and have concluded that, at this time, no other therapeutic approaches such as surgery, radiation therapy, or other chemotherapeutic treatments are clinically indicated as being more effective. At some later time, should these alternatives be clinically indicated, they will be discussed with you because this study does not preclude their use.

Consent for Autopsy

To fully evaluate the effects and safety of gene transfer, it will be necessary to obtain as much information as possible. In the event or occurrence of your death, evaluation of your organs will be a very valuable method to see the full effects of gene transfer. Please understand that we will therefore make a request for an autopsy from your next-of-kin in the event of your death from any cause, and we would encourage you to discuss this possibility with your family in advance.

Contraception

Because genetic material could circulate to multiple sites in the body, there is a small possibility that it could be unintentionally transmitted to unborn offspring where it could possibly have harmful effects. To avoid this possibility, you will be required to practice contraception for the 2-month duration of this program. To help us learn about the potential for this side effect, male participants will be asked to provide semen specimens for laboratory studies.

Other Pertinent Information

1. Confidentiality. When results of a study such as this are reported in medical journals or at meetings, the identification of those taking part is withheld. Medical records are maintained according to current legal requirements, and are made available for review, as required by the Food and Drug Administration or other authorized users, only under the guidelines established by the Federal Privacy Act. A qualified representative of the National Institutes of Health may inspect patient and study records. This procedure may attract attention from the media. We will make every effort to protect your confidentiality. Because of media interest, however, there is a significant chance that information concerning you and your treatment will appear publicly without your consent.
2. Policy regarding research-related injuries. In the unlikely event of physical injury resulting from research procedures, the University will provide first-aid medical treatment. Treatment of injuries or side effects directly related to this experimental treatment will be provided at no cost to you. Additional medical treatment will be provided in accordance with the determination by the University of its responsibility to provide such treatment. However, the University does not provide compensation to a person who is injured while participating as a subject in research. If you have questions regarding additional medical treatments, you may call the Patient Staff Relations Office (313/763-5456).
3. You will not be paid to take part in this study.
4. Outpatient and inpatient studies will be ordered to determine your eligibility for study and whether you have had a response to the treatment. Clinic visits, laboratory tests, x-rays, and scans will not be free. Items which are not covered by insurance which relate to this research protocol will be covered by research grants. The cost of tests and treatments unrelated to this study will be handled as usual and will depend on whether or not you have insurance and what costs your insurance covers. Unfortunately, insurance coverage cannot be guaranteed for all tests and treatments; however, you may discuss this issue with the hospital financial office or your insurance company before you agree to participate.
5. This consent form does not include consent relating to the risks of any surgical procedures. Any surgical procedures performed will require a separate consent form.

Please understand that you are free to withdraw your consent to participate in this study at any time during treatment or follow-up and seek care from any physician with no loss of benefits or disruption in your care.

The team of physicians and nurses providing care in this study are very experienced. You must realize, however, that unforeseeable or unexplained risks are always possible when investigational therapies are undertaken.

If you have questions pertinent to this research, you should contact Gary J. Nabel, M.D., Ph.D., at 313/747-4798.

If you feel that you have a research-related injury, contact Gary J. Nabel, M.D., Ph.D., at 313/747-4798.

The following numbers are for your use if medical problems develop during treatment:

Office: 313/747/4798
Clinical Research Center: 313/936-8090
Voice Mailbox: 313/764-9121 (after office hours)
Doctor or Nurse: 313/936-6266; Paul Watkins, M.D.
(This is a hospital beeper; ask the paging operator to page #9128. Use the following numbers in case of an Emergency Only).
(Outside of regular office hours if the paging service won't do.)
Home: 313/000-0000; Dr. Gary J. Nabel

Questions on my rights as a patient may be directed to Ann Munro in the Patient/Staff Relations Office at 313/763-5456.

I have fully explained to the patient, ________________, the nature of the treatment program described above and such risks as are involved in its performance.

____
Physician's Signature

I have been fully informed as to the procedures to be followed including those which are investigational, and have been given a description of the attendant discomforts, risk, and benefits to be expected, and the appropriate alternative procedures. I realize that, since my participation is voluntary, I can refuse this treatment without in any way prejudicing my future medical care. In signing this consent form, I agree to this method of treatment, and I understand that I will receive the best supportive care even if not receiving this protocol treatment. I also understand that my doctors can stop my treatment on this protocol if they feel the risks in my case have increased, over time, to exceed the potential benefits to me. I understand, also, that if I have any questions at any time, they will be answered. I have received a copy of this consent form.

I am not and will not become pregnant during this study (female participants). I will use contraception to avoid inducing a pregnancy while receiving treatments on this protocol (male and female participants).

I understand that the University will provide first-aid medical treatment in the unlikely event of physical injury resulting from research procedures. Treatment of injuries or side effects directly related to the experimental treatment will be provided at no cost to me. Additional medical treatment will be provided in accordance with the University's determination of its responsibility to do so. The University does not, however, provide compensation to a person who is injured while participating as a subject in research.

I have not engaged in any other research projects within the past six (6) months. [ ].

Within the past six (6) months, I have been involved in a study by Dr. ________________.

I have [ ] have not [ ] been under the care of a physician within the past twelve (12) months.

____
Signature of Patient

Date

Witness
7/93

16.0 Scientific Abstract

The goal of immunotherapy is to stimulate the immune system by modification of tumor cells or expansion of lymphocytes which respond specifically to tumor antigens. In this study, we will apply techniques of direct gene transfer to enhance the immune response against tumors *in vivo*. Patients with advanced cancer who have failed all effective therapy will be treated by injection of a DNA/liposome complex directly within the tumor. DNA will be used which encodes a heterodimeric cell surface protein recognized in the transplantation response. These genes include the HLA-B7 histocompatibility antigen and β-2 microglobulin gene in a non-viral eukaryotic expression vector plasmid. For this vector, a safe and effective dose to introduce this recombinant gene in HLA-B7$^-$ patients will be established. HLA-B7 expression will be confirmed *in vivo*, and the immune response stimulated by the expression of this antigen will be characterized. We will also determine whether this treatment facilitates tumor regression alone or in combination with other treatment modalities. This study will employ a similar strategy to our previous gene therapy protocol, but employs four improvements in technology, including more efficacious liposomes, optimized vector expression, catheter delivery and application to other several types of cancer. These studies will facilitate the development of other approaches, using different recombinant genes or in combination with cytokines or adoptive T cell therapy, to augment tumor immunity, and allow for greater potential efficacy. This method will also establish the safety of this non-viral approach to gene therapy, which could potentially be extended to treat a variety of other human diseases.

17.0 Non-technical Abstract

Many types of cancer cannot be cured by traditional medical treatments, including drugs, surgery, or radiation. In this study, an experimental treatment will be offered that may help to fight this disease. We will attempt to induce tumor regression by the introduction of genetic material that directs the synthesis of proteins which stimulate the immune system. The genetic material, DNA, will be introduced directly into the tumor by mixing it with fat bodies, or liposomes, and this mixture will be injected into the tumor or delivered by a catheter. The DNA will be taken into cells and cause them to produce proteins that stimulate tissue rejection. These proteins, called histocompatibility proteins, cause cells which contain it to be recognized as foreign by the immune system. In some cases, another immune stimulatory protein, interleukin-2, will also be included. The goal of the treatment is to stimulate the immune system to attack and kill the tumor. In this study, we will determine a safe and effective dose to administer the DNA liposome complex. Increasing amounts of this complex will be used in different patient populations. If no side effects are observed, repeated treatments will be instituted. The expression and nature of the immune response will also be characterized. This treatment may provide a therapeutic effect in cancer and could be applied to the treatment of other diseases.

21.0 Appendix IV—RESPONSE TO:

Points to Consider in the Design and Submission of Protocols for the Transfer of Recombinant DNA into the Genome of Human Subjects

PROTOCOL: Immunotherapy of Malignancy by *in Vivo* Gene Transfer into Tumors

PREPARED BY: Gary J. Nabel, M.D., Ph.D.

SUBMITTED TO: Human Gene Therapy Subcommittee
Recombinant DNA Advisory Committee
National Institutes of Health
Bethesda, Maryland 20892

I. Description of Proposal

A. Objectives and rationale of the proposed research. State concisely the overall objectives and rationale of the proposed study.

The aim of this proposal is to develop *in vivo* gene transfer approaches for the treatment of malignancy. We have previously performed a phase I study to analyze the toxicity and effects of this treatment. We now propose to include four modifications to improve gene delivery and expression *in vivo*. We will introduce a gene encoding an allogeneic human transplantation antigen, HLA-B7, into tumors *in vivo* and determine:

a) whether this recombinant gene transfer approach is safe in patients
b) a specific immune response is generated in response to this antigen
c) whether this stimulation results in rejection of the tumor

From the previous study, we find that this procedure is of minimal risk to the patient and could provide therapeutic effects in malignancy. An additional purpose of this study is to introduce improvements designed to improve its efficacy. These modifications will allow better gene delivery and will provide for the expression of MHC and cytokine genes in the microcirculation of tumors, which is likely to be of therapeutic benefit. It may also provide a general method to treat other human diseases. The studies in this protocol also provide the first step in allowing combination gene delivery for immunotherapy. Finally, some of the modifications in this approach, particularly catheter-based gene delivery, will provide information that could be applied to less incurable disorders, such as cardiovascular disease.

1. Use of recombinant DNA for therapeutic purposes.
   a. Why is the disease selected for treatment by means of gene therapy a good candidate for such treatment?

For several reasons, gene therapy represents a logical approach to the treatment of malignancy. Recently, it has become clear that the genetic instability of tumors leads to the synthesis of a variety of mutated products. Some of these genes contribute to the malignant phenotype. They also produce mutant proteins which can be recognized by the immune system. Since the tumors that will be treated are not responsive to other modes of treatment, there are no current alternative therapies which have been proven effective. The ability to express recombinant genes within these tumors provides a potent source of recombinant proteins which are synthesized locally and can have autocrine and paracrine effects which might not be achieved by systemic administration of recombinant growth factors. In addition, the expression of cell surface glycoproteins in a selected population of cells can stimulate a localized response which will minimize the potential damage to normal tissues. Since the approach which is being used involves improving the antigenicity of the tumor with a foreign MHC gene, it can be applied universally as a means to stimulate the immune system to recognize tumor-associated antigens relevant to the patient. Finally, the recombinant gene product expressed in these experiments does not directly stimulate cell proliferation, and its expression will cause the cells which express the recombinant vector to be eliminated. For this reason, long-term expression of the vector is a less major concern. In contrast to cytokine or lymphokine genes which stimulate cell proliferation and maintain expression *in vivo*, this treatment is therefore self-limited.

   b. Describe the natural history and range of expression in the disease selected for treatment. What objective and/or quantitative measures of disease activity are available? In your view, are the usual effects of the disease predictable enough to allow for meaningful assessment of the results of gene therapy?

The natural history and range of expression of malignancy is well known. Although there is individual variation in the progression of disease, a variety of objective measures are available, including evaluation by CT-scan, MRI, or other noninvasive markers. The additional objective measure of treatment in this experiment is the generation of a specific immune response against the foreign HLA-B7 antigen which has been introduced for treatment.

   c. Is the protocol designed to prevent all manifestations of the disease, to halt the progression of the disease after symptoms have begun to appear, or to reverse manifestations of the disease in seriously ill patients?

In initial experiments, the safety of direct gene transfer into tumors will be the primary goal of this study. Once the safety has been established, it is hoped that increasing doses of the recombinant gene, HLA-B7 and β-2 microglobulin, or these genes in combination with IL-2, may be used that will help to halt the progression of the disease or potentially reverse its progression either alone or in combination with other modes of treatment. These other modes of treatment include concomitant systemic administration of cytokines and adoptive T cell immunotherapy from draining lymph nodes. An important second aspect of this study will be to deliver the recombinant gene to sites of metastasis by catheterization. This approach could provide for direct gene transfer to sites of distant disease and could affect blood flow to the tumor.

   d. What alternative therapies exist? In what groups of patients are these therapies effective? What are their relative advantages and disadvantages as compared with the proposed gene therapy?

A variety of experimental protocols are being used to treat metastatic melanoma, adenocarcinoma, and other malignancies. These include the systemic administration of biological response modifiers, adoptive transfer using tumor-infiltrating lymphocytes, and antibody-mediated or immunotoxin cytolysis. None of these approaches have proven uniformly curative for malignancy, although limited success has been achieved (10%–30% partial remissions using various different protocols). We do not yet know the efficacy of the proposed treatment. There are two potential advantages of its use. First, by introducing the recombinant gene directly within the tumor, the intensity of the immune response can be maximized at the tumor site. Lymphocytes stimulated in this region will also be able to circulate to other sites. In tumors such as melanoma where the immune response may play an important role, this approach may well prove therapeutic. In addition, in the event that the procedure is not effective alone, it may be useful in combination with the other alternative immunotherapy strategies described above.

2. Transfer of DNA for other purposes.
   Into what cells will the recombinant DNA be transferred? Why is the transfer of recombinant DNA necessary for the proposed research? What questions can be answered by using the recombinant DNA?

The cells which will be modified by direct gene transfer *in vivo* include malignant cells, as well as normal vascular cells and fibroblasts which may be contained within the substance of the tumor and contribute to the microcirculation of the tumor. The transfer of the recombinant DNA is intended to stimulate an immune response within this region. The major questions that will be addressed by using this recombinant DNA are:

1) are there toxicities associated with these doses of DNA liposome complexes?
2) does this form of treatment induce a specific immune response against the recombinant gene expressed within the tumor?
3) is local inflammation within the tumor stimulated by this treatment?
4) does expression of this recombinant gene stimulate tumor regression

B. Research design, anticipated risks and benefits.
   1. Structure and characteristics of the biological system.
      Provide a full description of the methods and reagents employed for gene delivery and the rationale for their use. The following are specific points to be addressed:
      a. What is the structure of the cloned DNA that will be used?

The HLA-B7 gene has been inserted into a simplified eukaryotic expression vector which utilizes the RSV enhancer to stim-

ulate expression of the HLA-B7 gene. A second open reading frame is included using an internal ribosome initiation site derived from ECMV. This plasmid uses a bovine growth hormone poly A site, eliminating all viral processing and polyadenylation sequences. The plasmid DNA is grown in a standard *E. coli* host strain (DH 5α or XL1-Blue). This vector has been constructed by insertion of the HLA-B7 gene cDNA into the RSV β-globin plasmid (71,72). Briefly, the β-globin gene has been removed from this plasmid by digestion with Hind III and Bgl II treated with Klenow fragment of DNA polymerase and used as the backbone to insert the Bam HI to Sal I fragment of HLA-B7 treated with Klenow enzyme. This plasmid contains pBR322, a kanamycin resistance gene, the RSV enhancer, HLA-B7, ECMV ribosome entry site, β-2 microglobulin, and the bovine growth hormone polyadenylation sequence. The structures of this vector is also included in the appendix at the end of this section (not included in this publication).

The major difference between previous vector systems and that described in this protocol is the use of a non-viral vector. In the laboratory, plasmid DNA liposome complexes have been used in pre-clinical studies and have been shown to be safe and effective. Because it will not be introduced into a packaging cell line, it is likely to be at least as safe as standard retroviral vectors, since no other recombinant gene products can be incorporated into the delivery vehicle.

b. What is the structure of the material that will be administered to the patient?

(1) Describe the preparation, structure, and composition of the materials that will be given to the patient or used to treat the patient's cells.

The structure of the material that will be administered to the patient is a DNA liposome complex. This material is prepared at the time of instillation as follows: 10 minutes prior to delivery, 0.1 ml of plasmid DNA (0.05–50 mg/ml) is diluted to 200 μl with lactated-Ringer's solution at room temperature. 0.1 ml of DMRIE/DOPE liposome solution (0.15–15 mM). Each component will be stored separately in sterile vials and certified as acceptable by the FDA. The solution is left at room temperature for 5–10 minutes and 0.8 ml of sterile lactated-Ringer's is added to the liposome DNA solution. The optimal composition of the DNA liposome complex has been established for each batch by titration of DNA concentration and liposome concentration independently on human melanoma and renal cell carcinoma *in vitro*, and confirmed by direct injection into melanoma or other tumors *in vivo* prior to use. Each component, the liposome preparation and the DNA, will be tested for contaminants and toxicity and used according to previously established guidelines from the FDA. The liposome solution and DNA will be aliquoted in individual sterile vials mixed under sterile conditions as described above.

(2) Describe any other material to be used in preparation of the material to be administered to the patient. For example, if a viral vector is proposed, what is the nature of the helper virus or cell line? If carrier particles are to be used, what is the nature of these?

In addition to the plasmid DNA, the liposome solution will also be administered to the patient. This liposome consists of two lipids, including dioleolyl phosphatidylethanolamine (DOPE) and dimyristyloxypropyl-3-dimethyl-hydroxyethyl ammonium bromide (DMRIE). DOPE, DMRIE, DNA, and the DNA liposome compound is currently undergoing FDA review. As in our previous trial, separate certificates of analysis are used for each of these components. The DNA and liposome will be stored in sealed vials in a designated storage freezer and refrigerator prior to administration to the patient and reconstituted as described above in the Clinical Research Center.

2. Preclinical studies, including risk-assessment studies.

Describe the experimental basis (derived from tests in cultured cells and animals) for claims about efficacy and safety of the proposed system for delivery, and explain why the model(s) chosen is (are) the most appropriate.

a. Laboratory studies of the delivery system.

This direct gene transfer system has been studied extensively in the laboratory (1–5,19,21,22,69,73) using over 200 animals. These papers and the relevant preliminary studies are included under Section 13 of the proposal and in the attached manuscripts. The early conceptual basis for this work is described in the two *Science* manuscripts by Nabel et al. The MHC studies in the porcine animal model were described initially in the paper of Nabel et al. entitled "Transduction of a Foreign Histocompatibility Gene into the Arterial Wall Induces Vasculitis." The studies which pertain most directly to tumor protection are described in the paper by Plautz et al. entitled "Immunotherapy of Malignancy By *in Vivo* Gene Transfer into Tumors." Previous toxicity studies are also published, and a manuscript is being prepared regarding the previous human trial (74).

b. Laboratory studies of gene transfer and expression.

(1) What animal and cultured cell models were used in laboratory studies to assess the in vivo and in vitro efficacy of the gene transfer system? In what ways are these models similar to and different from the proposed human treatment?

The efficacy of this gene transfer approach has been studied in BALB/c mice using the CT26 transplantable colon carcinoma line. Recombinant genes have been delivered into cells and into tumor in the hind flank of these animals. Injections were performed similar to the method proposed for human treatment. Additional details and results of these studies in animals are described in the Clinical Protocol, Section 13. Studies are ongoing using human melanoma cells in nude mice as well.

(2) What is the minimal level of gene transfer and/or expression that is estimated to be necessary for the gene transfer protocol to be successful in humans? How was this level determined?

The level of gene transfer and expression required for successful treatment in humans is expected to be in the 1–5% range. This number has been derived using reporter genes, including HLA-B7 or β-galactosidase reporter genes in animal studies. Using this level of expression, successful cytolytic T cell responses have been generated in pigs and mice. These levels are determined by histochemical or immunohistochemical stains.

(3) Explain in detail all results from animal and cultured cell model experiments which assess the effectiveness of the delivery system (part 2.a. above) in achieving the minimally required level of gene transfer and expression (2.b.(2) above).

The background information regarding our general efforts to introduce recombinant genes by direct transfer *in vivo* is provided in the clinical protocol (Sections 1, 2, 13, Introduction, Objectives, and Preliminary Data). Using β-galactosidase as a marker, we have determined by histochemistry that ~1% of cells are routinely transfected by these procedures. Recently, we have begun to introduce class I MHC proteins to stimulate tissue rejection when transplanted into allogeneic or xenogeneic strains. To study this process and determine whether a foreign MHC gene can induce a rejection response, we have developed a model system in which the transplantation antigens are introduced directly into porcine vascular cells and into murine tumors. A retroviral vector encoding the human class I MHC gene, HLA-B7 (56), together with the neomycin resistance gene, was prepared in the PLJ vector, and amphotropic retroviral producer cell lines were derived (Clinical Protocol, Section 13, Fig. 1). A similar vector has been prepared for the mouse H-2K^{s} gene.

Using the method for direct gene transfer in vivo, the human class I MHC gene HLA-B7, or the BAG retroviral vector, was first introduced into localized segments of the iliofemoral artery in at least ten pigs. Expression of the HLA-B7 gene in vascular cells was confirmed by immunohistochemical staining, and the presence of plasmid DNA has been confirmed by PCR (19). Expression of HLA-B7 induces a pronounced inflammatory response in the localized arterial segment which is not observed in control animals infected with the retroviral vector encoding *E. coli* β-galactosidase (19). Inflammation is maximal at 17 days following transduction and subsides by 75 days (19) and is characterized by an accumulation of mononuclear cells and a granulomatous reaction. A similar response is seen when the plasmid DNA is introduced by lipofection with the new DMRIE/DOPE compound. Arterial segments from non-transduced vessels in animals where HLA-B7 has been introduced elsewhere has consistently shown no evidence of inflammation, showing that the primary immune response is restricted to the site of gene transfer, and this response is not seen with other growth factor genes (19,68,70). By immunohistochemical staining, responses are observed when ~1% of cells are transduced. Peripheral blood lymphocytes from pigs transduced with HLA-B7 also generate cytotoxic T cells which are specific for the introduced HLA-B7 antigen.

We have also transduced mouse tumors by direct injection into tumors *in vivo*. Gene transfer has been achieved using both retroviral vectors and DNA/liposome complexes. Tumors injected with β-galactosidase showed no alteration in growth, whereas animals injected *in vivo* with H-2K^{s} retrovirus showed markedly diminished tumor growth (5). Approximately 1% of cells at the site of injection are thought to express the recombinant gene. Splenic lymphocytes from this animal were analyzed using a ^{51}Cr release assay. In contrast to lymphocytes derived from CT26 mice injected with β-gal vectors, lymphocytes from H-2K^{s} injected mice demonstrate specific cytolytic reactivity for this antigen (5). More important, lymphocytes from H-2K^{s} but not control mice also became reactive against the parental unmodified tumor line. Based on these and other studies, a similar trial was undertaken in man using HLA-B7. In this case, no toxicity was observed and an anti-tumor CTL response was demonstrated in two patients where their tumor cell line could be grown.

(4) To what extent is expression only from the desired gene (and not from the surrounding DNA)? To what extent does the insertion modify the expression of other genes?

It is unlikely that other genes are directly affected by this gene transfer protocol. The safety issues regarding expression are similar to those which have been described in previous protocols using retroviral gene transfer. The approach used in this study is likely to be at least as safe as those employing retroviral vectors, which is complicated by the potential insertion of genetic material introduced from the packaging cell line. In the case of DNA liposome complexes, however, we have found that the DNA remains largely episomal but is quite stable, suggesting that the risk of insertional mutagenesis is lower. In over 200 animals evaluated, there have been no instances of malignancy arising that we have observed.

(5) In what percentage of cells does expression from the added DNA occur? Is the product biologically active? What percentage of normal activity results from the inserted gene?

Using different reporter genes, including β-galactosidase or HLA-B7, the percentage of cells containing recombinant DNA following liposome transfection is ~1%. The product is biologically active as determined by laboratory analysis of transduced cells as well as *in situ* immunohistochemical staining using a monoclonal antibody against the recombinant gene product (5,19). Levels of expression in cell lines transformed *in vitro*, normal levels of cell surface antigen are observed from expression of the inserted gene (Clinical Protocol, Section 13, Figs. 2,3).

(6) Is the gene expressed in cells other than the target cells? If so, to what extent?

It is likely that the gene can be expressed within tumor cells and in cells composing the microcirculation and interstitium of the tumor. Since these cells represent less than 1% of cells in the tumor, ~1% of these cells are transduced, and transduced cells are destroyed by the immune system, it is not anticipated that this effect will be harmful to the patient. It is hoped that inflammation within the tumor microcirculation will also provide a therapeutic effect.

c. Laboratory studies pertaining to the safety of the delivery/expression system.

The major safety issues regarding the delivery expression system center on the possibility of vector replication, cell transformation, and the possibility of creating uncontrolled inflammation. With respect to replication, the vector is a DNA liposome complex which cannot replicate. There has been no evidence of replication *in vivo* by PCR analysis of tissue specimens from animals. We have examined expression of DNA liposome complexes from over 30 mice, 20 rabbits, and 10 pigs injected with these complexes. When injected directly into the systemic circulation, plasmid is sometimes observed transiently in liver, kidney, heart, and brain by PCR, but this expression usually does not persist beyond 2 weeks (3,4). When injected

directly into the tumor, as proposed in this study, it has generally been confined to the site of injection, although it is occasionally found in other tissues. No PCR positivity has yet been detected in ovaries or testes, suggesting that it is unlikely to enter the germ line.

A second concern is the possibility of uncontrolled gene expression. Although high levels of expression might be obtained in any given cell, the vector is incapable of replication and limited to the site of expression, where it is eliminated by the immune system. It is therefore unlikely that this vector will pose a significant safety problem to the patient. With respect to the potential for insertional activation of oncogenes, this problem is likely to be less significant than for murine retroviral vectors. In the previous human trial, no toxicities were encountered.

3. Clinical procedures, including patient monitoring.

These are detailed in the clinical protocol. In addition to the protocol and patient monitoring, postmortem examinations will also be requested. These examinations will include not only pathologic examination of the tissue but PCR of different organs for the presence of the introduced plasmid DNA.

4. Public health considerations.

The public health hazard of direct liposomal transfection is considered less of a risk than the use of amphotropic retroviral vectors, which have been deemed by the National Institutes of Health to be of minimal public health risk, even in the event of accidental exposure. The potential to transmit DNA to the germ line is very low: examination of over 30 min and rabbits using PCR to detect plasmid within testes and ovaries has revealed no instances of PCR positivity even after direct intravenous injection.

5. Qualifications of investigators, adequacy of laboratory and clinical facilities.

Dr. Gary Nabel is an Associate Professor of Internal Medicine and Biological Chemistry with special expertise in retrovirology and the molecular biology of mammalian transcription factors. He has also trained clinically in Internal Medicine at the Brigham and Women's Hospital, Harvard Medical School, and is board certified in Internal Medicine. His current studies focus on direct gene transfer *in vivo* (in vascular cells and tumors) and the interaction of retroviruses, particularly HIV, with cellular factors that regulate viral gene expression.

Dr. Alfred Chang received his medical degree from Harvard Medical School and is currently professor of surgery at The University of Michigan Medical Center. Dr. Chang's expertise is in the field of surgical oncology and the experimental therapeutic treatment of malignancy using immunotherapy approaches. Dr. Chang trained in this area with Dr. Steven Rosenberg at the National Institutes of Health, and he has developed an independent program in this field at The University of Michigan Medical Center.

Dr. Elizabeth Nabel is an Associate Professor and Attending Physician in the Cardiac Catheterization Laboratory with extensive cardiology training. In addition, she has extensive laboratory experience in tissue culture of vascular smooth muscle cells, endothelial cells, and *in vivo* gene transfer of recombinant genes in studies performed at The University of Michigan Medical Center.

Dr. Gregory Plautz is Assistant Professor of Pediatric Hematology/Oncology and has developed a special expertise in retroviral gene expression and gene transfer, and has been an integral part of efforts in this laboratory to develop the gene transfer approaches described in this study. He has extensive experience with retroviral vectors, tumor inoculation and transfer, assays of immune function, and immunohistochemistry. As a lecturer in the Department of Pediatrics, he plans a career in academic medicine, and is board certified in Pediatrics and Pediatric Hematology/Oncology.

Dr. William Ensminger is Professor of Internal Medicine and a medical oncologist with a long-standing interest in the treatment of metastatic cancer. His special area of expertise is catheter delivery of drugs through the hepatic artery for metastatic liver disease. He will participate in the evaluation and treatment of patients with catheter-based gene delivery. Dr. Ensminger is also Associate Director of the Clinical Research Center. Dr. Kyung Cho, Director of Interventional Radiology with a special expertise in his area, will also participate in these studies.

Adequacy of the Laboratory and Clinical Facilities:

The Clinical Research Center is a dedicated clinical facility located on the 7th floor of The University of Michigan Medical Center Hospital. The Center is one of the largest GCRCs sponsored by the National Institutes of Health, with an annual budget in excess of $3.5 million and 9,500 square feet of dedicated space. There are 16 inpatient beds and 18 full-time nurses. Laboratory space includes 750 square feet for blood and specimen collection, a 1,000-square-foot research laboratory of the Center Director, Dr. Paul Watkins, and a 750-square-foot core dedicated to human gene therapy. The CRC also employs a Biostatistician and a Computer Systems Analyst to help in the interpretation and handling of data. In addition, there are other services which our CRC provides to facilitate studies. A lymphocyte immortalization core is located away from the patient care area in a 500-square-foot space in a different building. This facility performs EBV transformation of patient lymphocytes routinely. The CRC core human gene therapy laboratory space could be used to prepare liposomes and to perform *in vitro* analysis of immune function and immunocytochemistry.

C. Selection of patients.

See the Clinical Protocol, Section 3.

D. Informed consent.

The relevant informed consent form document is attached in the appendix to the clinical protocol. Included in the informed consent are the concerns regarding immune and HIV status in these individuals. Since the treatment will activate lymphocytes, and a concern has been raised about whether immune stimulation might accelerate the progression of AIDS, we have elected not to include HIV seropositive persons in this study.

E. Privacy and confidentiality.

We will follow standard procedures for communication with the press, including direct reporting and communication but no identification of the patient or details of their medical problems other than those obvious from the entry criteria. Thus, we will make every attempt to preserve the privacy and confidentiality of the patient. The informed consent protocol explains this policy to the patient and includes a caveat that breaches could occur through aggressive reporting that is beyond our control,

although every effort will be made to protect the patient's privacy and confidentiality.

II. Special Issues

A. What steps will be taken, consistent with point I.E. above, to ensure that accurate and appropriate information is made available to the public with respect to such public concerns as may arise from the proposed study?

These issues will be dealt with as described in Section 1E. The Public Relations Department at The University of Michigan Medical Center will also help to coordinate interactions with the press and public.

B. Do you or your funding sources intend to protect under patent or trade secret laws either the products or the procedures developed in the proposed study?

No, although patents have been filed on procedures developed in animal studies, we have no plans to restrict access to information derived from these studies. They will be available for public review.

III. Requested Documentation
—included in the Clinical Protocol document

A. Clinical Protocol
B. University of Michigan Medical Center IRB and minutes (Clinical Protocol, Appendix I, Section 18)
C. Scientific abstract (Clinical Protocol, Section 16)
D. Non-technical abstract (Clinical Protocol, Section 17)
E. CV (Curricula Vitae) (Clinical Protocol, Appendix II, Section 19)
F. Other federal agencies in this review: FDA
G. Other material: published articles (see Clinical Protocol, Appendix III, Section 20)

IV. Reporting Requirements

Any adverse effects of a treatment will be reported as required. Progress reports will be made to the Recombinant DNA Advisory Committee and the Gene Therapy Subcommittee at 6-month intervals or upon request.